3.0 CHARACTERIZATION OF SUBSTANCES TESTED IN *IN VITRO* AR TA ASSAYS

3.1 Introduction

In vitro AR TA assay data were collected for a total of 145 substances that had been evaluated in assays that measured the ability of a substance to activate or inhibit transcription of androgen-inducible genes. As shown in **Table 3-1**, 68 of these substances had been tested for both agonism and antagonism, while 51 had been tested for agonism only, and 20 for antagonism only. Seventeen substances had been tested in a cell proliferation assay, and of these, two had also been tested for agonism, eight for both agonism and antagonism, one for antagonism and six for neither agonism nor antagonism.

Table 3-1 Distribution of Substances by Type of *In Vitro* AR TA Assay

Type of TA Assay	Number of Substances
Agonism and Antagonism	68
Agonism	51
Antagonism	20
Agonism and Cell Proliferation	2
Agonism, Antagonism and Cell Proliferation	8
Antagonism and Cell Proliferation	1
Cell proliferation	6

The data were obtained from 26 peer-reviewed, scientific journal articles and one report containing unpublished data. The majority of these studies used DHT as the reference androgen; however, other reference androgens including R1881 (five publications), testosterone (four publications), and mibolerone (one publication) were also used.

Relevant information on the substances tested (i.e., chemical name, Chemical Abstract Service Registry Number [CASRN], chemical supplier or source, and purity) was extracted from the publications and entered into a database. Some publications did not include all of this information. For publications in which only chemical structures were provided, every effort was made to identify the names and CASRN of the substances tested. CASRNs were obtained from various sources, including the National Library of Medicine's ChemID database and *The Merck*

Index. However, no attempt was made to determine the source and purity of test substances if this information was not provided in the publication. Different publications often used a unique chemical name for the same substance. When this occurred, the most commonly used chemical name was chosen and assigned to the substance, regardless of the chemical name used in a particular publication, and the unique chemical nomenclature was entered into the database as a synonym (**Appendix C**).

3.2 Rationale for Selection of Substances/Products Tested in *In Vitro* AR TA Assays

Many of the substances tested in *in vitro* AR TA assays were selected to address basic research questions regarding the nature of AR binding and transcriptional activation processes. Mechanistic studies investigating the steps involved in AR activation or inhibition of target genes used both naturally-occurring steroids (e.g., DHT, testosterone, androstenedione, and 17β-estradiol) and synthetic AR agonists and antagonists (e.g., oxandrolone, fluoxymesterone, hydroxyflutamide, and cyproterone acetate). Some of these substances, particularly the natural androgens and synthetic anti-androgens, were studied to obtain a better understanding of their different potencies and biological activities. Some of the synthetic anti-androgens (e.g., hydroxyflutamide and bicalutamide) have been investigated in AR TA studies to evaluate their mechanisms of action as therapeutic agents, and to determine why some of these substances have both agonist and antagonist activities. In addition, some substances were investigated to determine which derivative (e.g., norethisterone and 11-ketonorethisterone) or metabolites of a specific substance (e.g., DDE and dihydroxy-DDE) enhanced or inhibited AR-induced transcriptional activation.

During the last decade, with the growing concern about endocrine disruptors, some of these substances (e.g., vinclozolin and its major metabolites, *o,p'*-DDT and its major metabolites, atrazine, kepone, and linuron) were tested in AR TA assays to identify substances that may act as androgen agonists or antagonists in humans and wildlife. Most of the publications that reported AR TA assay data on industrial chemicals, pesticides, and environmental contaminants tested these substances to evaluate their potential to disrupt the endocrine system. Typically, these publications also reported on the utility of a particular AR TA assay as a screen for endocrine disruptor activity.

3.3 Chemical and Product Classes Tested

Chemical and product class information for the substances tested in *in vitro* AR TA assays is provided in **Appendix C**. Substances were assigned to chemical classes based on available information from standardized references (e.g., *The Merck Index*) and from an assessment of chemical structure. As shown in **Table 3-2**, the chemical classes that have been tested most extensively in *in vitro* AR TA assays are nonphenolic steroids, organochlorines, phenolic steroids, and polycyclic aromatic hydrocarbons. Of the 145 substances included in this BRD, 17 could be assigned to two chemical classes.

Table 3-2 Chemical Classes Tested in *In Vitro* AR TA Assays

Chemical Classes	Number of Substances
Alcohol	1
Alkyl sulfonate	1
Alkylphenol	4
Anilide	3
Aromatic amine	1
Azole	1
Benzophenone	1
Biphenyl	1
Bisphenol	2
Carboxylic acid	1
Coumarin	1
Dioxin	1
Diphenolalkane	2
Ether	3
Glucuronic acid	2
Imidazole	5
Imide	1
Indene	1
Isoflavone	1
Lactone	2
Nitrile	5

Chemical Classes	Number of Substances
Organochlorine	30
Organothiophosphate	2
Phenol	6
Phenyl ether	1
Phthalate	4
Polychlorinated biphenyl	3
Polycyclic aromatic hydrocarbon	10
Pyrethrin	4
Pyrimidine	2
Resorcylic acid lactone	1
Steroid, nonphenolic	35
Steroid, phenolic	12
Stilbene	4
Sulfonylurea	1
Triazine	1
Triphenylethylene	3
Urea	3

Product classes were assigned based on information from *The Merck Index* and the National Library of Medicine's ChemFinder. Only a few product classes are represented, as shown in **Table 3-3.** The most common product classes tested in *in vitro* AR TA assays are pharmaceuticals and pesticides. Of the substances included in this BRD, 21 had no known commercial use, so were not classified within a product class.

Table 3-3 Product Classes Tested in *In Vitro* AR TA Assays

Product Classes	Number of Substances
Adhesive	1
Buffer	1
Chemical intermediate	12
Coating	1
Dielectric fluid	3
Dye	1
Natural product	6
Pesticide (includes metabolites, derivatives, and degradation products)	45
Pharmaceutical (includes metabolites)	60
Plasticizer	3
Preservative	2
Unclassified	21